CHEMICAL CONSTITUENTS OF THE NECTARS OF TWO *ERYTHRINA* SPECIES AND THEIR HYBRID[1]

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Abstract

In an analysis of sugars, amino acids, and other substances (lipids, antioxidant organic acids, phenolics, alkaloids, proteins) of nectar, the hybrid *Erythrina* × *bidwillii* showed a quantitative intermediacy and qualitative additiveness in amino acids compared to its parents, *E. herbacea* and *E. crista-galli*, and intermediacy in sugars and the other compounds.

In two recently published papers (Baker & Baker, 1976a, 1977) we have shown that the floral nectar amino acid complements of closely related species in several genera of flowering plants are intraspecifically surprisingly constant, although usually interspecifically different. In the F_1 hybrids between the species, the complements are additive on a qualitative basis (although not necessarily so quantitatively). Indirect evidence has been accumulated that shows genetic segregation in subsequent hybrid generations, and the inheritance of amino acid production is under experimental study in *Geranium* and *Silene*.

In two other papers (Baker, 1978; Baker & Baker, 1980) we have shown that the relative proportions of the three common sugars in nectars (sucrose, glucose and fructose) from a wide range of species are, to some extent, determined by the taxonomic affinities of the species concerned, but also show adaptation to the type of pollinator whose services are used by that species. However, we have not been able to find evidence in the literature as to the inheritance of nectar sugar characteristics. This lacuna, together with the fact that none of our studies of amino acid inheritance has involved trees, suggested that attention should be given to the chemical constituents from *Erythrina*. The present paper is a preliminary study which it is hoped may become more nearly comprehensive in the future.

For *Erythrina* we have provided evidence that the relative proportions of sucrose and hexose sugars in the nectars of some hummingbird-pollinated species (viz., high sucrose:hexose ratios) are different from those of some other species where pollination is by passerine birds (viz., low sucrose:hexose ratios) (Baker, 1978; Baker & Baker, 1980; see also Feinsinger & Bolten, this symposium). The demonstration of this pollinator-related difference was foreshadowed in the data we provided for Cruden & Toledo (1977) in their comparison of *E. coralloides* A. DC. (hummingbird pollinated) with *E. breviflora* A. DC. (pollinated by orioles and tanagers). We also showed (Baker, 1978) that the complement of amino acids in *E. breviflora* was larger, and included all of the "essential" amino acids, as well as being more concentrated.

On the basis of the sugar proportions in its nectar (Baker & Baker, 1980) and

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an absence of reliable observations of hummingbird pollination of trees in their native South America (see Toledo, 1974), we suggested (Baker & Baker, 1980) that *Erythrina crista-galli* L. may also turn out to be pollinated by passerine birds. Consequently, it is of interest to compare nectar analyses of this species with those of a species that is a member of the same genus and known to be pollinated by hummingbirds (Toledo, 1974). This is *Erythrina herbacea* L. Although placed in separate subgenera by Krukoff & Barneby (1974), these species can be hybridized. They have the same chromosome number, $2n = 42$ (Atchison, 1947).

With woody plants such as the erythrinas, it has not been feasible to make artificial hybrids for the special purpose of chemical analyses of their nectars. Consequently, advantage was taken of the existence of a horticultural hybrid between these species, *Erythrina* × *bidwillii* Lindley. Although this hybrid may have been made more than once since its original production by J. C. Bidwill over a century ago (Krukoff & Barneby, 1974), there is no evidence that generations after the F_1 have been raised, propagation being by rooting of cuttings. Consequently, we believe that the tree of *E.* × *bidwillii* growing in the University of California Botanical Garden in Berkeley, is very probably an F_1 hybrid. The accession cards at the Garden indicate that the tree (#50.1853) was received from the horticultural firm of Evans and Reeves, Los Angeles, in 1950. A tree of *E. crista-galli* also grows in the U.C.B. Garden (#34.489). It is derived from seeds, collected in South America, by Dr. T. H. Goodspeed. The identities of these trees were checked with descriptions in the latest monograph of the genus (Krukoff & Barneby, 1974) and compared with herbarium specimens. Our own herbarium specimens are in UC.

Graham & Tombs (1974) report that *Erythrina* × *bidwillii* has approximately 76% good pollen while the figure for *E. crista-galli* is about 88%. Our plants gave similar estimates.

Nectars from these trees were collected in the Botanical Garden in 1977 and 1978 and nectar of *Erythrina herbacea* subsp. *herbacea* was collected from a wild plant growing in a "hammock" within the Fairchild Tropical Garden, Coral Gables, Florida, in April 1978 by Dr. J. B. Fisher. There can be no doubt as to its identity as, in addition to its subshrubby habit, this is the only species growing wild in Florida. These nectar samples were immediately spotted onto chromatography paper, dried and used for analyses.

Methods of Analysis

SUGARS

Analyses of the sugars in these nectars were carried out by the method described by Baker & Baker (1980). In summary, it is a single direction, descending paper chromatographic analysis, using n-propanol:ethyl acetate:water mixture as a solvent, with staining by oxalic acid in ethanol mixed with p-aminobenzoic acid in chloroform and acetic acid. After drying and heating, the chromatograms are examined under U.V. illumination, in which all sugars fluoresce. The amounts of each sugar are estimated by eluting the individual sugar spots with methanol and measuring their fluorescence in a filter fluorometer. Calibration curves for

TABLE 1. Proportions of sugars in nectars of two *Erythrina* species and their hybrid.

Sugar	*E. crista-galli*[a] (1977)	*E. crista-galli*[a] (1978)	*E. crista-galli*[a] (1978)	*E. crista-galli*[a] (1978)	*E.* × *bidwillii*[b] (1977)	*E.* × *bidwillii*[b] (1978)	*E. herbacea*[c] (1978)
Melezitose	N.D.[d]	0.009	0.007	0.008	N.D.[d]	0.014	0.017
Maltose	0.010	0.017	0.017	0.014	N.D.[d]	N.D.[d]	N.D.[d]
Sucrose	0.031	0.033	0.034	0.028	0.172	0.182	0.394
Glucose	0.396	0.464	0.406	0.403	0.450	0.421	0.318
Fructose	0.553	0.476	0.533	0.546	0.378	0.384	0.272
Ratio sucrose:hexoses	0.032	0.035	0.037	0.030	0.208	0.226	0.668

[a] Four determinations
[b] Two determinations
[c] One determination
[d] N.D. = not detected

each sugar correlating fluorescence with amounts of the sugar present are then used to estimate the proportions of the sugars present in the nectar.

AMINO ACIDS

Amino acid complements were identified and the relative proportions of each amino acid present in each nectar were estimated by the dansylation–U.V. fluorescence method described in detail by Baker & Baker (1976a, 1976b).

OTHER SUBSTANCES

Qualitative tests for lipids (OsO_4 test), antioxidant organic acids (2,6-dichlorophenol-indophenol test), phenolics (p-nitraniline test) and alkaloids (iodoplatinate and Dragendorff tests) were also made. Proteins were tested for by the brom-phenol blue method. For details of these test methods see Baker & Baker (1975), except for the test for phenolics which follows the method of Gray, Thorpe and White (Smith, 1969: 434).

RESULTS

SUGARS

The sugar analyses (Table 1) show a consistent pattern of hexose dominance in *Erythrina crista-galli*, sucrose richness (but not dominance) in *E. herbacea*, and an intermediate picture in *E.* × *bidwillii*. Also intermediate is the showing of the trisaccharide melezitose (which is slightly more concentrated in *E. herbacea*). The disaccharide maltose was hard to detect in *E. crista-galli* and could not be detected in *E.* × *bidwillii* and *E. herbacea*.

AMINO ACIDS

The amino acid analyses (Table 2) show that both species produce nectar with a large number of amino acids (21 for *Erythrina crista-galli*; 20 for *E. herbacea*). However, there are slight differences in the complements: *E. herbacea* lacks methionine in our analysis and the two species have different "unknown" amino acids (presumably of a "nonprotein" nature). *Erythrina* × *bid-*

TABLE 2. Amino acid complements of two *Erythrina* species and their hybrid. The proportions of each acid in the total for each taxon are shown.

Amino acid	*E. crista-galli*	*E. × bidwillii*	*E. herbacea*
Alanine	0.101	0.089	0.039
Arginine	0.069	0.028	0.004
Asparagine	0.065	0.111	0.174
Aspartic	0.006	0.024	0.036
Cysteine, etc.	0.003	0.019	0.018
Glutamic	0.006	0.047	0.038
Glutamine	0.202	0.177	0.231
Glycine	0.016	0.029	0.054
Histidine	0.012	0.008	0.025
Isoleucine	0.065	0.044	0.043
Leucine	0.017	0.017	0.036
Lysine	0.025	0.028	0.032
Methionine	0.014	0.018	N.D.[a]
Phenylalanine	0.023	0.039	0.027
Proline	0.075	0.075	0.076
Serine	0.074	0.058	0.028
Threonine	0.071	0.041	0.024
Tryptophan	0.010	0.022	0.029
Tyrosine	0.019	0.037	0.009
Valine	0.098	0.067	0.058
Unknown #1	0.013	0.006	N.D.[a]
Unknown #2	N.D.[a]	0.018	0.022

[a] N.D. = not detected

willii shows every one of the amino acids recorded for each plant (22 total) in the usual qualitative "additive" pattern for F_1 hybrids. For most of the individual amino acids the results for *E. × bidwillii* are also intermediate quantitatively (as measured by proportional representation).

OTHER SUBSTANCES

Once again, *Erythrina × bidwillii* shows an intermediate picture (Table 3). The test for lipids gave a negative result for nectars from all three taxa. Organic (reductive) acids were not detectable in *E. crista-galli* or the hybrid, but gave a strong reaction for *E. herbacea*. *Erythrina herbacea* and *E. × bidwillii* provided convincing evidence of the presence of phenolics while these were apparently only slightly represented in *E. crista-galli*. Proteins were not detectable in any of the nectars.

TABLE 3. Representations of various chemicals in nectars of two species of *Erythrina* and their hybrid.

Chemical	*E. crista-galli*	*E. × bidwillii*	*E. herbacea*
Lipids	N.D.[a]	N.D.[a]	N.D.[a]
Organic acids	Negative	Negative	Strong
Phenolics	Slight	Positive	Positive
Alkaloids	Negative	Negative	Positive
Proteins	N.D.[a]	N.D.[a]	N.D.[a]

[a] N.D. = not detected

DISCUSSION

The results reported here refer to only one plant of each species. It can hardly be doubted that if a wider sampling of many populations of each species were undertaken (and it is our ambition to do this for the genus *Erythrina* as a whole) a greater amount of intraspecific variation might be revealed. However, the results are consistent with data that we have obtained from other investigations (Baker & Baker, 1976a, 1977).

There is intermediacy in the representation of the sugars in the hybrid and both quantitative intermediacy and qualitative additiveness in the amino acid complements.

Among the individual amino acids, the strengths of the amines, glutamine and asparagine, are notable, but the two "nonprotein" amino acids are probably the most important in their suggestion, being one in each species, that "nonprotein" amino acids in nectar could reveal taxonomic and phylogenetic information of value in studying the genus *Erythrina*. Certainly, there must be a wider investigation of nectar amino acids to see if there is merit to this suggestion.

It is interesting that the modest amount of information about the presence or absence of organic acids, phenolics and alkaloids suggests a greater degree of "chemical protection" against nectar-robbers in the nectar of *Erythrina herbacea* compared with *E. crista-galli*. Again, this is a promising area for investigation on a genus-wide basis.

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